Vol. 31, No. 5, December 1993 BIOCHEMISTRY and MOLECULAR BIOLOGY INTERNATIONAL
Pages 911–922

PURIFICATION AND CHARACTERIZATION OF THE BRANCHED CHAIN α-KETOACID DEHYDROGENASE COMPLEX FROM *SACCHAROMYCES CEREVISIAE.*

David A. Sinclair[1,2], Ian W. Dawes[1] and J. Richard Dickinson[2]

[1]School of Biochemistry and Molecular Genetics, University of New South Wales, PO Box 1, Kensington, NSW 2033, Australia
[2]School of Pure and Applied Biology, University of Wales College of Cardiff, PO Box 915, Cardiff CF1 3TL, U.K.

Received November 3, 1993

SUMMARY: Branched chain α-ketoacid dehydrogenase complex was purified from *Saccharomyces cerevisiae* by polyethylene glycol fractionation and chromatography on Sephacryl S-200, DEAE-cellulose and Sepharose CL-2B. Electrophoresis on sodium dodecyl sulfate-polyacrylamide gels indicated the enzyme contained subunits of M_r = 57,000, 52,000, 47,000 and 38,000. The specific activity of the purified enzyme was 0.82 μmol NADH/min/mg protein at 30°C with 16 mM α-ketoisovalerate as substrate. The apparent K_m values for α-ketoisovalerate, α-ketoisocaproate and α-keto-β-methylvalerate were 21, 22, and 20 mM, respectively. The preparation was also able to oxidize the intermediates of threonine and methionine metabolism, α-keto-γ-methiolbutyrate and α-ketobutyrate, with K_m values of 13 and 8 mM, respectively.

INTRODUCTION

The initial stages of catabolism of branched chain amino acids in *Saccharomyces cerevisiae* is similar to that found in mammals in that it occurs via the branched chain α-ketoacid dehydrogenase complex (EC 1.2.4.4) (1), not via the "Ehrlich Pathway" as first proposed (2, 3). The reaction involves the oxidative decarboxylation of the branched chain α-ketoacids derived from transamination of valine, leucine and isoleucine. The mammalian enzyme is distinct from the related multi-enzyme complexes, pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, whereas in the prokaryotic *Bacillus subtilis* the reaction is catalysed by a bifunctional pyruvate dehydrogenase (4). Wild-type strains of *Escherichia coli* do not utilise the pathway (5).

There has been widespread interest in mammalian branched chain α-ketoacid metabolism since lesions in the enzyme results in maple syrup urine disease (6). The

1039-9712/93/170911-12$05.00/0
Copyright © 1993 by Academic Press Australia.
All rights of reproduction in any form reserved.

enzyme complex has been purified from a number of mammals and shown to consist of four subunits, E1α (M_r = 46,000), E1β (M_r = 38,000), E2 (M_r = 52,000) and a weakly associated E3 (M_r = 49,000 - 55,000) (7, 8, 9). The E1 component, comprised of E1α and E1β subunits, uses thiamine pyrophosphate as a cofactor to catalyse the oxidative decarboxylation of the branched chain α-ketoacids. The E2 (acyltransferase) uses a covalently bound lipoate to transfer the branched chain acyl group to coenzyme A. The E3 (lipoamide dehydrogenase) with a covalently bound FADH reoxidises reduced lipoate using NAD^+ as substrate. In *Saccharomyces cerevisiae*, lipoamide dehydrogenase is encoded by a single gene, *LPD1*, whose product acts in at least two other complexes: pyruvate dehydrogenase and α-ketoglutarate dehydrogenase (10, 1).

The regulation of the activity of the mammalian branched chain α-ketoacid dehydrogenase complex occurs primarily via a specific protein kinase and a specific phosphatase. The kinase often copurifies with the E1α subunit and can be shown to inactivate the purified complex by incubation with Mg^{2+} and ATP *in vitro* (11). The mammalian pyruvate dehydrogenase complexes also contain small amounts of a pyruvate dehydrogenase kinase and a pyruvate dehydrogenase phosphatase which regulate the activity of the E1 subunit. The existence of such a mechanism for yeast pyruvate dehydrogenase has yet to be shown conclusively (12).

In this paper we report the purification of branched chain α-ketoacid dehydrogenase from *Saccharomyces cerevisiae*. The final enzyme preparation was composed of at least four distinct polypeptides which were similar in size to those of the subunits of the mammalian branched chain α-ketoacid dehydrogenase complex. We also present kinetic data for various substrates including the branched chain α-ketoacids, α-keto-γ-methiolbutyrate and α-ketobutyrate.

METHODS

Materials. Mauri "Pinnacle" compressed Baker's yeast Mauri Products Ltd., Kingston-upon-Hull, U.K.) was used as the source of enzyme. Sephacryl S-200 and Sepharose CL-2B were purchased from Pharmacia LKB (Milton Keynes, U.K.). Centricon-100 microconcentrators (>100,000 MW cutoff) were the product of Amicon Ltd., Stonehouse, U.K.). Diethylaminoethyl-cellulose (DE-52) was purchased from Whatman, Maidstone, UK. Molecular weight protein standards, porcine heart lipoamide dehydrogenase, substrates, and other chemicals were purchased from Sigma Chemical Co. (Poole, U.K.).

Buffer A consisted of 50 mM potassium phosphate buffer (pH 7.4 at 4°C), 0.1 mM EDTA, 0.2 mM thiamine pyrophosphate, 0.2 mM phenylmethylsulfonyl fluoride. YEPG growth medium contained yeast extract (10 g/l), bacteriological peptone (20 g/l) and 3% (v/v) glycerol.

Enzyme assays. Branched chain α-ketoacid dehydrogenase activity was assayed spectrophotometrically at 30°C as described previously (1) except for kinetic studies on the purified enzyme for which NAD^+ was substituted for 3-acetylpyridine adenine dinucleotide. Specific activities are expressed as μmol NAD^+ or 3-acetylpyridine adenine dinucleotide reduced/min/mg protein. Protein concentration was determined by the Bradford (1976) method (13).

Kinetic values were calculated using the program "Enzyme Kinetics" for an Apple Macintosh. The branched chain α-ketoacid dehydrogenase kinase assay was performed with 1 mM $MgCl_2$ and 0.8 mM ATP (14) in the following buffers: phosphate buffer (50 mM potassium phosphate pH 7.4); triethanolamine buffer (50 mM triethanolamine-HCl, pH 7.4); and Tris-acetate buffer (50 mM Tris-base adjusted to pH 7.4 with acetic acid).

Electrophoresis. Samples for electrophoresis were heated to 100°C for 5 min in 2% (w/v) sodium dodecylsulfate, 0.005% (w/v) bromophenol blue, 10% (v/v) glycerol and TTS buffer (25 mM Tris pH 7.5; 250 mM tricine; 0.1% SDS). Electrophoresis was performed at 100 mV for 4 to 6 h on 10% to 20% (w/v) gradient polyacrylamide gels containing TTS buffer (Gradipore Ltd., Pyrmont, Australia). Proteins were visualised by either Coomassie blue or silver-staining methods.

RESULTS

Purification of branched chain α-ketoacid dehydrogenase complex. Commercial compressed yeast (300 g) was incubated with shaking for 12 h at 30°C in 4 l of YEPG medium (in a total flask volume of 8 l) containing 100 mg/l penicillin. YEPG medium was chosen since this medium causes the induction of high specific activity of the branched chain α-ketoacid dehydrogenase (1). Cells were harvested, washed in sterile water, and resuspended in 100 ml of buffer A. All procedures thereafter were performed at 0-4°C. Cells were disrupted by homogenising 30 ml aliquots with an equal volume of glass beads in a Braun homogeniser for 1 min at maximum setting. The homogenate was centrifuged at 12,000 x *g* for 15 min to pellet large cell debris. By dropwise addition of a 50% (w/v) stock solution, the supernatant was made to 2% (w/v) polyethylene glycol 8,000, and the mixture was stirred for 10 min. After centrifugation at 12,000 x *g* the supernatant was made to 5% (w/v) in polyethylene glycol and centrifuged as above. This step precipitated the pyruvate dehydrogenase and α-ketoglutarate dehydrogenase complexes which are often difficult to separate from the branched chain α-ketoacid dehydrogenase complex due to their similar sizes. The supernatant was then brought slowly to 9% (w/v) in polyethylene glycol and centrifuged as above.

The pellet obtained was resuspended in approximately 3 ml buffer A and applied to a Sephacryl S-200 column equilibrated with the same buffer. The branched chain α-ketoacid dehydrogenase complex was eluted in peak III whereas the larger

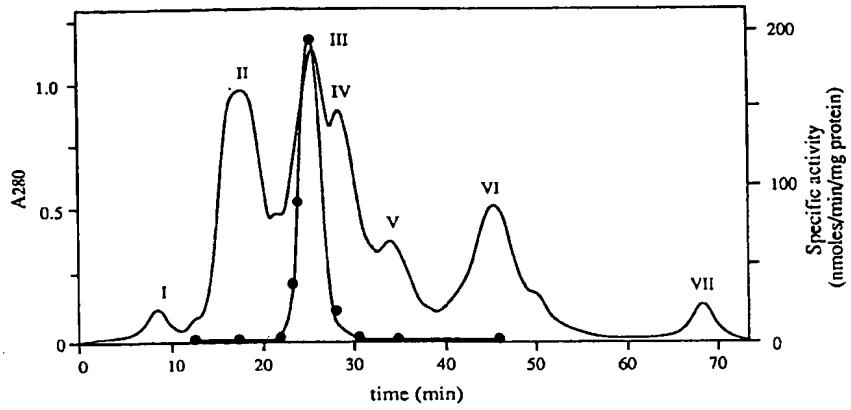

Fig. 1. Elution profile of size-exclusion chromatography of branched chain α-ketoacid extract. Partially purified polyethylene glycol fractionated extract was loaded onto a Sephacryl S-200 columm (400 x 15 mm) pre-equilibrated with Buffer A. Fractions were eluted at a rate of 0.4ml/ min and assayed for branched chain α-ketoacid (shaded circles). The appropriate fractions were pooled and concentrated with size exclusion membranes (M_r cutoff 100,000).

molecules such as glycogen were eluted in the initial peak (Fig. 1). Since centifugation at 170,000 x *g* resulted in complete loss of branched chain α-ketoacid dehydrogenase activity, the appropriate fractions were combined and concentrated with a microconcentrator (with an M_r cutoff of 100,000) at 1,000 x *g* for 1 h .

The solution (250 µl) was applied to a DEAE-cellulose column equilibrated with Buffer A. The column was then washed extensively with the same buffer (3 column volumes) until no further protein was eluting as detected from the absorbance at 280 nm. A second wash with Buffer A containing 0.05% (w/v) NaCl resulted in the passage of a brown band at the solvent front containing branched chain α-ketoacid dehydrogenase activity (Fig. 2). The appropriate fraction (approximately 2 ml) was concentrated as before.

The eluate was then applied to a Sepharose CL-2B column equilibrated with Buffer A. Two main peaks of protein were obtained. The second larger peak eluted as brown fractions and showed branched chain α-ketoacid dehydrogenase activity. The fractions corresponding to this peak were pooled and concentrated as before and the resulting activity could be stored in Buffer A for at least 3 weeks at -70°C without noticable loss in specific activity.

The purification of the branched chain α-ketoacid dehydrogenase complex is summarised in Table 1. The specific activity of the final preparation was 0.55 nmol

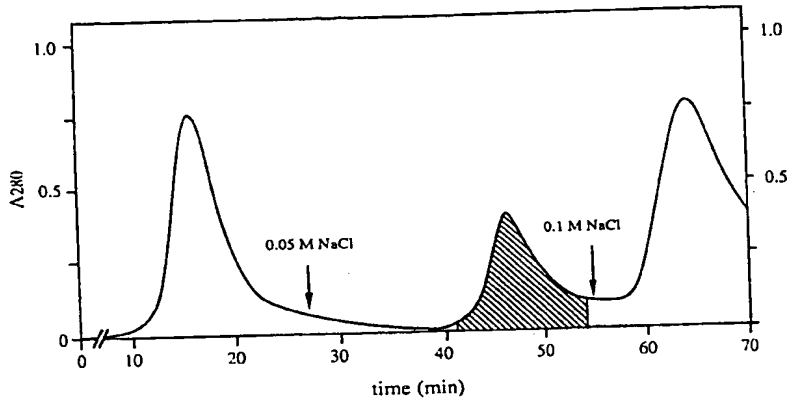

Fig. 2. Elution profile of anion-exchange chromatography of purified extract. Purified fractions obtained by size-excusion chromatography were pooled and loaded on a DEAE-cellulose (100 x 0.7 mm) column equilibrated with Buffer A (pH 7.4). The column was washed extensively with buffer until no further proteins were eluting. The column was washed with Buffer A containing 0.05 M NaCl and the fraction containing highly purified branched chain α-ketoacid complex was collected (shaded area).

acetylpyridine adenine dinucleotide reduced/min/mg protein using 16 mM α-ketoisovaleric acid as substrate. The final overall yield was 6%.

Characterisation of the purified branched chain α-ketoacid dehydrogenase complex. Most procedures for the purification of branched chain α-ketoacid dehydrogenase from higher eukaryotes resulted in the loss of the E3 subunit (lipoamide dehydrogenase). This resulted in preparations that exhibited only low activity for the complex and required the addition of exogenous lipoamide dehydrogenase to the assay. In this study, addition of purified bovine lipoamide dehydrogenase to either the crude or purified preparations did not affect enzyme activity implying that the yeast lipoamide dehydrogenase was still present. Furthermore, the color of the preparation was indicative of that seen in preparations of lipoamide dehydrogenase due to the bound FAD cofactor.

SDS-polyacrylamide gel electrophoresis of the complex (Fig. 3) showed that this preparation contained four polypeptides with a pattern almost identical to that of purified branched chain α-ketoacid dehydrogenase from other eukaryotes (15, 7). The two smaller polypeptides (M_r = 38,000 and 47, 000) correspond to the E1α and E1β subunits of the branched chain α-ketoacid dehydrogenase complex, respectively. The polypeptide of M_r = 52,000 corresponds the dihydrolipoyl transferase subunit (E2) and

Table 1. Purification of branched chain α-ketoacid dehydrogenase from *Saccharomyces cerevisiae*.

Purification stage	Total protein mg	Specific activity[a] µmol/min/mg protein	Total activity[a] µmol/min	Yield %
Clarified homogenate[b]	9350	0.015	140	100
5% PEG supernatant	6050	0.018	108	77
9% PEG pellet	630	0.130	82	59
Sepharose S-200 eluate	144	0.16	23	16
DEAE-cellulose eluate	23	0.65	15	11
Sepharose CL-2B	14	0.82	7.8	6

[a]Activities were determined using 0.8 mM α-ketoisovaleric acid as substrate
[b]Refers to supernatant after initial 12,000 x *g* spin

that of M_r = 57,000 corresponds to lipoamide dehydrogenase (E3). The value for the lipoamide dehydrogenase is in reasonable agreement with the value of M_r = 56,000 obtained by Kresze and Ronft (16) from SDS gel electrophoresis. The *S. cerevisiae* gene encoding lipoamide dehydrogenase has been cloned and sequenced, and assuming no glycosylation, gives a calculated M_r for the FAD-containing monomer of 54,730 (17). A fifth polypeptide of M_r = 62,000 that co-purified with the complex remains unidentifed, although the relative molecular mass is reminiscent of the large subunit of the 'SP' (spermine activated) phosphatase of pyruvate dehydrogenase (18).

The apparent V_{max} value for α-ketoisovalerate was more than twice that of the other two major substrates, α-ketoisocaproate and α-keto-β-methyl valerate, however the K_m values for all three substrates were almost identical (Table 2). The reciprocal plot of velocity versus substrate concentration was linear up to 16 mM substrate, above which inhibition by all three of the above substrates was observed. As noted for the bovine enzyme (19), the complex will use α-keto-γ-methiolbutyrate and α-ketobutyrate as substrates. Comparison of the relative V_{max} (in which the V_{max} for α-ketoisovalerate is normalised) shows an identical figure for a-ketobutyrate for both the yeast and bovine kidney enzymes, but α-keto-γ-methiolbutyrate is not utilised to anything like the same extent in yeast. It should also be noted that the K_m values for the yeast enzyme are approximately five hundred-fold greater than the mammalian enzyme for all substrates tested.

Unlike the other components of the branched chain α-ketoacid dehydrogenase assay, omission of CoA did not affect the activity of the enzyme in the preparation.

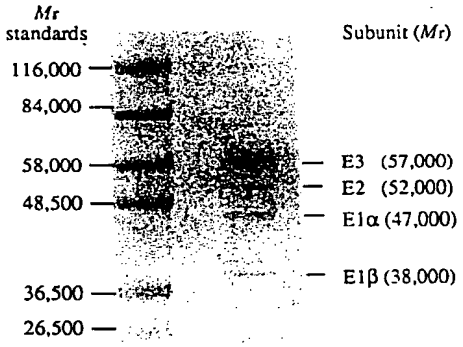

Fig. 3. SDS-polyacrylamide gel electrophoresis of the branched chain α-ketoacid dehydrogenase preparation from Sepharose CL-2B (table 1). The protein sample (15 μg) was added to 2% SDS (w/v), 0.005% (w/v) bromophenol blue, 10% (v/v) glycerol and TTS buffer (25 mM Tris pH 7.5; 250 mM tricine; 0.1% (w/v) SDS) and heated to 100°C for 5 min. Electrophoresis was performed with 10% to 20% (w/v) gradient TTS buffered polyacrylamide gels at 100 mV for 4 to 6 h. Proteins were visualised by Coomassie blue and destained in a 5% (v/v) methanol/ 5% (v/v) acetic acid solution.

This implies either there was residual CoA in the protein preparation or the cofactor was tightly associated with the complex. However, extensive dialysis of the branched chain α-ketoacid dehydrogenase preparation for 12 hours against Buffer A (containing 2 μM thiamine pyrophosphate) did not result in a requirement for CoA in the branched chain α-ketoacid dehydrogenase assay. Addition of valine (0.5 mM or 5 mM) to the assay did not activate the branched chain α-ketoacid dehydrogenase complex as described for *Pseudomonas putida* branched chain α-ketoacid dehydrogenase complex (9, Table 3).

The presence of a branched chain α-ketoacid dehydrogenase kinase, observed as a decrease in branched chain α-ketoacid dehydrogenase activity, could not be detected by incubation with ATP (Table 3) as demonstrated with other branched chain α-ketoacid dehydrogenase complex preparations purified from various animal tissues (20, 11). We cannot, however, exclude the possibility that the kinase did not copurify with the complex or that the majority of the complexes were already in an inactive state.

Table 2. Kinetic parameters for substrates of branched chain α-ketoacid metabolism.

Substrate	*Km* mM	*V*max μmoles/min/mg protein	Relative*V*max
α-Ketoisovalerate	21.3 ± 1.4[a]	19.9 ± 0.8	100
α-Ketoisocaproate	21.8 ± 1.7	7.5 ± 0.4	38
α-Keto-ß-methylvalerate	19.9 ± 0.5	7.6 ± 0.1	38
α-Ketobutyrate	7.5 ± 0.9	4.0 ± 0.4	20
α-Keto-γ-methiolbutyrate	13.3 ± 1.2	1.6 ± 0.2	8
NAD^+	35.3 ± 2.1	0.2 ± 0.0	N/A

[a]Values are means ± SE
[b]Parameters determined using 0.8 mM α-ketoisovaleric acid as substrate

Table 3. Conditions used in assay for the detection of branched chain α-ketoacid dehydrogenase kinase.

Pretreatment of branched chain α-ketoacid dehydrogenase complex[a]	Specific activity μmol/min/mg protein
No ATP	3.0
Buffer A + 0.8 mM ATP	2.9
Buffer A + 30 mM ATP	3.0
Buffer A + 0.8 mM ATP + 10 mg/ ml BSA	3.9
Triethanolamine buffer	2.7
Triethanolamine buffer + 0.8 mM ATP	2.8
Tris-acetate buffer	1.8
Tris-acetate buffer + 0.8 mM ATP	2.2

[a] Enzyme was incubated in buffer for 1 hour and assayed in Buffer A with 16 mM α-ketoisovalerate.

DISCUSSION

Until recently it was thought that the catabolism of branched chain amino acids in yeast proceeded via the "Ehrlich Pathway" (2). However, given the functional similarities of yeast and higher eukaryotes, an investigation was initiated to determine whether there were greater similarities in this pathway than had been suspected (21). Dickinson and Dawes (1992) demonstrated that the pathway proceeds via a branched chain α-ketoacid dehydrogenase and this study describes the purification and kinetic characterization of the enzyme complex responsible for this activity.

By a combination of polyethylene glycol precipitation, size exclusion and anion exchange chromatography, the branched chain α-ketoacid dehydrogenase of *Saccharomyces cerevisiae* was obtained in high purity. Both the absence of a M_r = 42,000 band and pyruvate dehydrogenase activity from the branched chain α-ketoacid dehydrogenase preparation indicated that pyruvate dehydrogenase was successfully removed by the purification procedure. The purified preparation was composed of at least 4 polypeptides which have estimated relative molecular masses of 57,000, 52,000, 47,000 and 38,000. The gene encoding the yeast E3 subunit has been cloned and sequenced, yielding a predicted M_r of 54,730 (17), in reasonable agreement with the size of the polypeptide (M_r = 57,000) obtained by SDS electrophoresis. The sizes of these polypeptides are in close agreement with those published for the bovine branched chain α-ketoacid dehydrogenase: E3 (M_r = 55,000), E2 (M_r = 52,000), E1α (M_r = 46,000), and E1β (M_r = 37,000) (7, 8). The similarity between the two strongly indicates that yeast branched chain α-ketoacid dehydrogenase is a multi-subunit complex related to that of higher eukaryotes, however, identification of the subunits will depend on specific assays for these activities.

In *Saccharomyces*, the lipoamide dehydrogenase E3 component is common to other α-ketoacid dehydrogenases including pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, and the *lpd1* mutation leads to the simultaneous loss of these two complexes and the branched chain α-ketoacid dehydrogenase activity (10, 1). This raises the interesting question of how the expression of the *LPD1* gene is regulated, given the requirement of its product (lipoamide dehydrogense) to act in three different complexes whose expression is dependent on different stimuli.

The purified yeast branched chain α-ketoacid dehydrogenase preparation catalysed the oxidative decarboxylation of α-ketoisovalerate, α-ketoisocaproate and α-keto-β-methylvalerate. As reported for the bovine branched chain α-ketoacid dehydrogenase (19), the preparation also was capable of recognising the intermediates of threonine and methionine metabolism, α-ketobutyrate and α-keto-γ-methiolbutyrate respectively. Hence the branched chain α-ketoacid dehydrogenase is implicated in the

catabolism of at least five amino acids, although the very low activity towards α-keto-γ-methiolbutyrate might indicate that branched chain α-ketoacid dehydrogenase plays only a minor role in the catabolism of methionine to mercaptans in yeast. Presumably trans-sulfuration to α-ketobutyrate is the more important route. In any case, intracellular pools of methionine are always very low in yeast (22) and hence methionine will usually be re-utilized rather than degraded.

The Michaelis constants for the substrates used here were greater than those from mammals. This difference may reflect the relative toxicities of the branched chain α-ketoacids in higher eukaryotes and yeast. In humans, this toxicity manifests itself as neurological damage in individuals with defective branched chain α-ketoacid dehydrogenase. However, in microorganisms including yeast and bacteria, the branched chain α-ketoacids probably reach higher intracellular concentrations when the organisms are catabolizing branched chain amino acids. In *Pseudomonas putida*, for example, the K_m for α-ketoisovaleric acid is 460 μM (9) which is mid way between the values for yeast and bovine kidney. In *Bacillus subtilis* the dual purpose pyruvate dehydrogenase and branched chain α-ketoacid dehydrogenase enzyme has a K_m of 1.3 mM for α-ketoisovaleric acid (4).

The possibility remains that the complex may require an activator for optimal activity and this was missing from the assay. In *P. putida* addition of 5 mM L-valine to the assay reduced the Michaelis constant of branched chain α-ketoacid dehydrogenase by an order of magnitude (9). However, addition of L-valine to both the crude yeast extract or the purified branched chain α-ketoacid dehydrogenase preparation did not affect either the maximum velocity or the Michaelis constant of the enzyme. Despite a well characterised mechanism for inactivition by phosphorylation of the mammalian branched chain α-ketoacid dehydrogenase, we found no evidence for the existence of such a mechanism modifying the yeast enzyme. It is, however, possible that the yeast branched chain α-ketoacid dehydrogenase kinase does not copurify with the enzyme or that in crude extracts it is difficult to detect, as has been shown for the putative yeast pyruvate dehydrogenase kinase (12).

ACKNOWLEDGEMENT

This work was supported by the Australian Department of Industry, Trade and Commerce Grant No. 91/8325.

REFERENCES

1. Dickinson, J. R., and Dawes, I. W. (1992) The catabolism of branched-chain amino acids occurs via 2-oxoacid dehydrogenase in *Saccharomyces cerevisiae*. J. Gen. Microbiol. 138, 2029-2033.

2. Ehrlich, F. (1904) Über das natürliche isomere des leucins. Berichte der Deutschen Chemischen Gesellschaft. 37, 1809-1840.
3. Neubauer, O., and Fromherz, K. (1911). Uber den Abbau der Aminosäuren bei der Hefegärung. Hoppe-Seyler's Z. Physiol. Chem. 70, 326-350.
4. Lowe, P. N., Hogdson, J. A., and Perham, R. N. (1983) Dual role of a single multienzyme complex in the oxidative decarboxylation of pyruvate and branched-chain amino acids in *Bacillus subtilis*. Biochem. J. 215, 133-140.
5. Sykes, P. J., Burns, G., Menard, J., Hatter, K., and Sokatch, J. R. (1987). Molecular cloning of genes encoding branched-chain keto acid dehydrogenase of *Pseudomonas putida*. J. Bacteriol. 169, 1619-1625.
6. Danner, D. J., and Elsas L. J. (1989) in *The Metabolic Basis of Inherited Disease*. (Scriver, C. R. ed.) pp. 671-692, McGraw-Hill, NY.
7. Pettit, F. H., Yeaman, S. J., and Reed, L. J. (1978) Purification and characterization of branched-chain α-ketoacid dehydrogense complex from bovine kidney. Proc. Natl. Acad. Sci. USA 75, 4881-4885,
8. Danner, D. J., Lemmon, S. K., Besharse, J. C., and Elsas, L. J. (1979) Purification and characterization of branched-chain α-ketoacid dehydrogenase from bovine liver mitochondria. J. Biol. Chem. 254, 5522-5526.
9. Sokatch, J. R., McCully, V., and Roberts, C. M. (1981) Purification of a branched-chain keto acid dehydrogenase from *Pseudomonas putida*. J. Bacteriol. 148, 647-652.
10. Dickinson, J. R., Roy, D. J., and Dawes, I. W. (1986) A mutation affecting lipoamide dehydrogenase, pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase activities in *Saccharomyces cerevisiae*. Mol. Gen. Genet. 204, 103-107.
11. Paxton, R., and Harris, R. A. (1982) Isolation of rabbit liver branched chain α-ketoacid dehydrogenase and regulation by phosphorylation. J. Biol. Chem. 257, 14433-14439.
12. Uhlinger, D. J., Yang, C. and Reed, L. J. (1986) Phosphorylation-dephosphorylation of pyruvate dehydrogenase from bakers' yeast. Biochemistry 25, 5673-5677.
13. Bradford, M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-254.
14. Fatania, H. R., Lau, K. S., and Randle, P. J. (1981) Inactivation of purified ox kidney branched chain 2-oxoacid dehydrogenase complex by phosphorylation. FEBS Letters 132, 285-288.
15. Shimomura, Y., Paxton, R., Ozawa, T., and Harris, R. A. (1987) Purification of branched chain α-ketoacid dehydrogenase complex from rat liver. Anal. Biochem. 163, 74-78.
16. Kresze, G., and Ronft, H. (1981) Pyruvate dehydrogenase complex from baker's yeast: molecular structure, dissociation, and implications for the origin of mitochondria. Eur. J. Biochem.119, 581-587.
17. Ross, J., Reid, G. A., and Dawes, I. W. (1988) The nucleotide sequence of the *LPD1* gene encoding lipoamide dehydrogenase in *Saccharomyces cerevisiae*: comparison between eukaryotic and prokaryotic sequences for related enzymes and identification of potential upstream control sites. J. Gen. Microbiol. 134, 1131-1139.
18. Damuni, Z., and Reed, L. J. (1987) Purification and characterization of a divalent cation-independent spermine-stimulated protein phosphatase from bovine kidney. J. Biol. Chem. 262, 5133-5138.

19. Jones, M. A., and Yeaman, S. J. (1986) Oxidative decarboxylation of 4-methylthio-2-oxobutyrate by branched-chain α-ketoacid dehydrogenase complex. Biochem. J. 237, 621-623.
20. Lau, K. S., Fatania, H. R., and Randle, P. J. (1981) Inactivation of rat liver and kidney branched chain α-ketoacid dehydrogenase complex by adenosine triphosphate. FEBS Letters 126, 66-70.
21. Dickinson, J. R., and Norte, V. (1993). A study of branched-chain amino acid aminotransferase and isolation of mutations affecting the catabolism of branched-chain amino acids in *Saccharomyces cerevisiae.* FEBS Letters 326, 29-32.
22. Jones, E. W., and Fink, G. R. (1982) in *The Molecular biology of the yeast Saccharomyces cerevisiae.* (Strathern, J. N., Jones, E. W., and Broach, J. R., ed.) pp. 181-299, Cold Spring Harbor Laboratory Press, N. Y.